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FOR MORE INFORMATION
Yizhi Jane Tao, Department of Biochemistry and Cell Biology, Rice University
ytao@rice.edu

Crystal Structure of the Influenza A Virus Nucleoprotein Reveals Architectural Details of the Viral Ribonucleoprotein Complex

Q. Ye and Y.J. Tao

Department of Biochemistry and Cell Biology, Rice University

The segmented, negative-sense RNA genome of the influenza virus is packaged in the form of ribonucleoprotein complexes (RNP), which contain not only the viral RNA, but also multiple copies of the viral nucleoprotein and a viral RNA polymerase. Besides forming the protein scaffold of the viral RNP, the nucleoprotein, one of the most abundant proteins in infected cells, has also been shown to interact with a large number of viral and host factors. The crystal structure of NP provides insights into the high-order structure of the RNP and into how NP may interact with cellular importin and the viral polymerase.

The genome of the influenza virus is organized into eight ribonucleoprotein (RNP) complexes, each of which contains one RNA segment bound to the viral nucleoprotein (NP) at a stoichiometry of one NP per 24 nucleotides, thus forming a rod-shaped, double-helical hairpin structure. The two RNA termini in each RNP are associated with a trimeric viral polymerase at one end of the RNP (**Figure 1**).

To elucidate the architectural details of the RNP, especially how NP interacts with both NP and viral RNA, our laboratory undertook crystallographic studies of the influenza A virus NP. Recombinant nucleoprotein, expressed in either insect cells or *E. coli*, were purified as oligomers of different sizes. Despite the size heterogeneity, NP from the influenza virus A/WSN/33 was crystallized in the space group of $C2_12_12_1$ with one NP trimer per asymmetric unit. Using multiple isomorphous replacement and anomalous scattering (MIRAS), the structure of NP was solved at 3.2 Å resolution.

The structure of NP has the shape of a PacMan with a head and a body domain (**Figure 2**). The polypeptide, which starts in the body domain, winds back and forth between the two domains three times, giving rise to a topology different from that of the rhabdovirus nucleoprotein. In the back of the molecule is a 28aa tail loop, which is inserted inside a neighboring NP and plays an important role in NP-NP interaction in an NCS trimer. Due to flexible linkers between the tail loop and the rest of the NP structure, the same tail loop interaction is observed among the three NCS-related NP, although the rotational angles relating the three subunits varies from 110º to 126º. Indeed, we have found that an inter-subunit salt bridge in the tail loop is essential for NP oligomerization.

The structure of NP, with its calculated electrostatic potential, shows that the large groove between the head and the body domain is likely to be the RNA binding site. Interestingly, this groove is facing the exterior, different from the situation in the rhabdoviruses, where RNA is bound to the interior of the NP oligomeric ring. The RNA binding site of the influenza virus NP contains amino acids widely distributed in sequence, explaining why previous attempts to identify a single RNA-binding domain were not successful. In addition, the first 20aa stretch, which contains a nuclear localization sequence (NLS), is located on the outer surface of the NP trimer and is disordered, allowing importin-α binding and further nuclear import.

Therefore, the crystal structure of NP provides direct evidence that RNA is exposed on the external surface of the influ-

Qiaozhen Ye

Yizhi Jane Tao

enza virus RNPs (**Figure 1**). The tail loop of NP likely mediates the lateral interaction of neighboring NP molecules, which are likely to be slightly offset along the symmetry axis to facilitate the formation of a continuous helix. Because of the tail loop linker flexibility, the same NP-NP interaction can be preserved when the double-helical RNP is unwound to loose polymers during RNA synthesis.

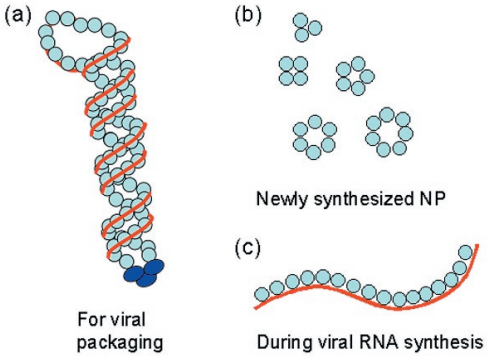

Figure 1. *NP in different functional states. (a) RNP. (b) Newly made NP proteins. (c) Loose NP-RNA polymer. NP molecules are shown by blue circles, RNA by red lines, and viral polymerase by dark-blue ovals.*

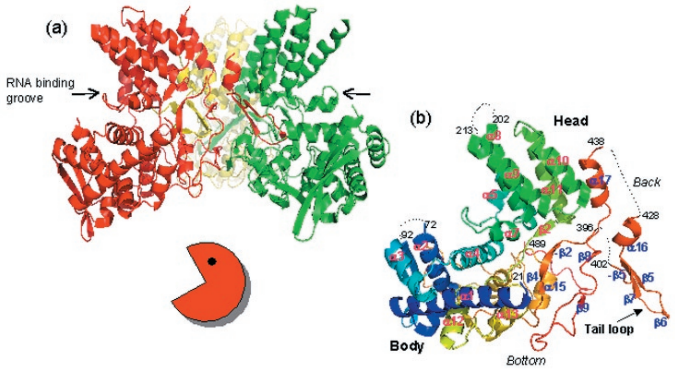

Figure 2. *NP crystal structure. (a) An NCS trimer with three subunits colored differently. (b) The red subunit from (a) colored continuously from N-terminus in blue to C-terminus in red. The disordered termini are marked by amino acid numbers.*